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L7 ANSWER 1 OF 9 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN AN 2008:490739 BIOSIS

9 DUPLICATE REMOVE L6 (14 DUPLICATES REMOVED)

DN PREV200800490738

PROCESSING COMPLETED FOR L6

- TI High throughput production of recombinant human proteins for crystallography.
- AU Gileadi, Opher [Reprint Author]; Burgess-Brown, Nicola A.; Colebrook, Steve M.; Berridge, Georgina; Savitsky, Pavel; Smee, Carol E. A.; Loppnau, Peter; Johansson, Catrine; Salah, Eidarus; Pantic, Nadia H.
- SO Kobe, B [Editor]; Guss, M [Editor]; Huber, T [Editor]. Methods in Molecular Biology, (2008) pp. 221-246. Methods in Molecular Biology, Publisher: HUMANA PRESS INC, 999 RIVERVIEW DR, STE 208, TOTOWA, NJ 07512-1165 USA. Series: METHODS IN MOLECULAR BIOLOGY. ISSN: 1064-3745. ISSN: 978-1-58829-809-6(H).

DT Book; (Book Chapter)

- LA English
- ED Entered STN: 3 Sep 2008
 - Last Updated on SIN: 3 Sep 2008
- AB This chapter presents in detail the process used in high throughput bacterial production of recombinant human proteins for crystal structure determination. The core principles are: (1) Generating at least 10 truncated constructs from each target gene. (2) Ligation-independent cloning (LIC) into a bacterial expression vector. All proteins are expressed with an N-terminal, TEV protease cleavable fusion peptide. (3) Small-scale test expression to identify constructs producing soluble protein. (4) Liter-scale production in shaker flasks. (5) Purification by Ni-affinity chromatography and gel filtration. (6) Protein characterization and preparation for crystallography. The chapter also briefly presents alternative procedures, to be applied based on specific knowledge of protein families or when the core protocol is unsatisfactory. This scheme has been applied to more than 550 human proteins (>10,000 constructs) and has resulted in the deposition of 112 unique structures. The methods presented do not depend on specialized equipment or robotics; hence, they provide an effective approach for handling individual proteins in a regular research laboratory

L7 ANSWER 2 OF 9 MEDLINE on STN DUPLICATE 1

- AN 2008374571 MEDLINE
- DN PubMed ID: 18542867
- TI High throughput production of recombinant human proteins for crystallography.
- AU Gileadi Opher; Burgess-Brown Nicola A; Colebrook Steve M; Berridge Georgina; Savitsky Pavel; Smee Carol E A; Loppnau Peter; Johansson

- Catrine; Salah Eidarus; Pantic Nadia H
- CS The Structural Genomics Consortium, Botnar Research Centre, University of Oxford, Oxford, UK.
- Methods in molecular biology (Clifton, N.J.), (2008) Vol. 426, pp. 221-46. SO Journal code: 9214969, ISSN: 1064-3745,
- CY United States
- Journal; Article; (JOURNAL ARTICLE) DT LA English
- FS
- Priority Journals
- EM 200808
- ED Entered STN: 11 Jun 2008 Last Updated on STN: 19 Aug 2008 Entered Medline: 18 Aug 2008
- AB This chapter presents in detail the process used in high throughput bacterial production of recombinant human proteins for crystal structure determination. The core principles are: (1) Generating at least 10 truncated constructs from each target gene. (2) Ligation-independent cloning (LIC) into a bacterial expression vector. All proteins are expressed with an N-terminal, TEV protease cleavable fusion peptide. (3) Small-scale test expression to identify constructs producing soluble protein. (4) Liter-scale production in shaker flasks. (5) Purification by Ni-affinity chromatography and gel filtration. (6) Protein characterization and preparation for crystallography. The chapter also briefly presents alternative procedures, to be applied based on specific knowledge of protein families or when the core protocol is unsatisfactory. This scheme has been applied to more than 550 human proteins (>10,000 constructs) and has resulted in the deposition of 112 unique structures. The methods presented do not depend on specialized equipment or robotics; hence, they provide an effective approach for handling individual proteins in a regular research laboratory
- ANSWER 3 OF 9 MEDLINE on STN

DUPLICATE 2

- 2008067110 MEDLINE AN
- PubMed ID: 18221021 DN
- TI Crystallization and preliminary X-ray analysis of fluorescent protein mBanana
- AU Zhou Yangbin; Wu Yifeng; Song Jiaping; Ding Yu; Hu Xiaojian; Zhang Zhihong CS Department of Physiology and Biophysics, Fudan University, 200433 Shanghai
- so Protein and peptide letters, (2008) Vol. 15, No. 1, pp. 113-4. Journal code: 9441434. ISSN: 0929-8665.
- CY Netherlands
- DT Journal: Article: (JOURNAL ARTICLE)
 - (RESEARCH SUPPORT, NON-U.S. GOV'T)
- LA English FS
- Priority Journals
- EM 200805
- ED Entered STN: 29 Jan 2008
 - Last Updated on STN: 10 May 2008 Entered Medline: 9 May 2008
- mBanana is a novel monomeric red fluorescent protein mutant. It was AB cloned and expressed in Escherichia coli with 10 histidine residues at its N-terminal. After cleavage of the His tag by TEV protease, the mBanana was further purified and crystallized by the hanging-drop vapor-diffusion technique. The crystals can diffract to 2.0A resolution and one set of completed data was collected. It showed that the orthorhombic mBanana crystal was in space group P21 with
 - unit cell parameters (48.629, 42.667, 61.714, 90, 111.676, 90) and contained one molecule in one asymmetric unit.

- AN 2005028473 MEDLINE DN PubMed ID: 15654889
- ΤТ Comparison of the substrate specificity of two potyvirus proteases.
- Tozser Jozsef; Tropea Joseph E; Cherry Scott; Bagossi Peter; Copeland AU Terry D; Wlodawer Alexander; Waugh David S
- Department of Biochemistry and Molecular Biology, Research Center for Molecular Medicine, University of Debrecen, Debrecen, Hungary... tozser@indi.biochem.dote.hu
- SO The FEBS journal, (2005 Jan) Vol. 272, No. 2, pp. 514-23. Journal code: 101229646. ISSN: 1742-464X.
- CY England: United Kingdom
- DT (COMPARATIVE STUDY)
 - Journal; Article; (JOURNAL ARTICLE)
- LA English FS
- Priority Journals
- EM 200503
- ED Entered STN: 19 Jan 2005
 - Last Updated on STN: 2 Mar 2005
- Entered Medline: 1 Mar 2005 AB The substrate specificity of the nuclear inclusion protein a (NIa)
- proteolytic enzymes from two potyviruses, the tobacco etch virus (TEV) and tobacco vein mottling virus (TVMV), was compared using oligopeptide substrates. Mutations were introduced into TEV protease in an effort to identify key determinants of substrate specificity. The

specificity of the mutant enzymes was assessed by using peptides with complementary substitutions. The crystal structure of

TEV protease and a homology model of TVMV protease were

used to interpret the kinetic data. A comparison of the two structures and the experimental data suggested that the differences in the specificity of the two enzymes may be mainly due to the variation in their

S4 and S3 binding subsites. Two key residues predicted to be important for these differences were replaced in TEV protease with the corresponding residues of TVMV protease. Kinetic analyses of the

mutants confirmed that these residues play a role in the specificity of the two enzymes. Additional residues in the substrate-binding subsites of TEV protease were also mutated in an effort to alter the specificity of the enzyme.

DUPLICATE 4

- ANSWER 5 OF 9 MEDLINE on STN
- AN 2005293963 MEDITINE
- PubMed ID: 15919091 DN
- ΤI Crystal structure of tobacco etch virus protease shows the protein C terminus bound within the active site.
- ΑU Nunn Christine M; Jeeves Mark; Cliff Matthew J; Urquhart Gillian T; George Roger R; Chao Luke H; Tscuchia Yugo; Djordjevic Snezana
- Department of Biochemistry and Molecular Biology, University College CS London, Gower Street, London, WC1E 6BT, UK.
- Journal of molecular biology, (2005 Jul 1) Vol. 350, No. 1, pp. 145-55. SO Journal code: 2985088R. ISSN: 0022-2836.
- England: United Kingdom
- DT Journal; Article; (JOURNAL ARTICLE) (RESEARCH SUPPORT, NON-U.S. GOV'T)
- LA English
- FS Priority Journals
- EM 200507
- ED Entered STN: 8 Jun 2005
 - Last Updated on STN: 20 Jul 2005 Entered Medline: 19 Jul 2005
- AB Tobacco etch virus (TEV) protease is a cysteine protease exhibiting stringent sequence specificity. The enzyme is widely used in biotechnology for the removal of the affinity tags from

recombinant fusion proteins. Crystal structures of two TEV protease mutants as complexes with a substrate and a product peptide provided the first insight into the mechanism of substrate specificity of this enzyme. We now report a 2.7A crystal structure of a full-length inactive C151A mutant protein crystallised in the absence of peptide. The structure reveals the C terminus of the protease bound to the active site. In addition, we determined dissociation constants of TEV protease substrate and product peptides using isothermal titration calorimetry for various forms of this enzyme. Data suggest that TEV protease could be inhibited by the peptide product of autolysis. Separate modes of recognition for native substrates and the site of TEV protease self-cleavage are proposed.

DUPLICATE 5

- L7 ANSWER 6 OF 9 MEDLINE on STN
- AN 2003524095 MEDLINE

DN PubMed ID: 14601399

- TI [Tobacco etch virus proteinase: crystal structure of the active enzyme and its inactive mutant]. Proteinaza virusa gravirovki tabaka: kristallicheskaia struktura aktivnogo fermenta i eco neaktivnogo mutanta.
- AU Zhdanov A S; Phan J; Evdokimov A G; Tropea J E; Kapust R B; Li M; Wlodawer A; Waugh D S
- CS Macromolecular Crystallography Laboratory, Center for Cancer Research, National Cancer Institute at Frederick, MD 21702-1201, United States. zdanov@ncifcrf.gov
- SO Bioorganicheskaia khimiia, (2003 Sep-Oct) Vol. 29, No. 5, pp. 457-60. Journal code: 7804941. ISSN: 0132-3423.
- CY Russia: Russian Federation
- DT (ENGLISH ABSTRACT)
- Journal; Article; (JOURNAL ARTICLE)
- LA Russian
- FS Priority Journals
- EM 200403
- ED Entered STN: 7 Nov 2003 Last Updated on STN: 2 Mar 2004
- Entered Medline: 1 Mar 2004 AB Tobacco Etch Virus Protease (TEV protease)
 - is widely used as a tool for separation of recombinant target proteins from their fusion partners. The crystal structures of two mutants of TEV protease, active autolysis-resistant mutant TEV-S219D in complex with the proteolysis product, and inactive mutant TEV-C151A in complex with a substrate, have been determined at 1.8 and 2.2 A resolution, respectively. The active sites of both mutants, including their oxyanion holes, have identical structures. The C-terminal residues 217-221 of the enzyme are involved in formation of

the binding pockets S3-S6. This indicates that the autolysis of the peptide bond Met218-Ser219 exerts a strong effect on the fine-tuning of the substrate in the enzyme active site, which results in considerable decrease in the enzymatic activity.

- L7 ANSWER 7 OF 9 CAPLUS COPYRIGHT 2008 ACS on STN
- AN 2003:784050 CAPLUS
- DN 140:159556
- TI Tobacco etch virus protease: Crystal structure of the active enzyme and its inactive mutant
- AU Zdanov, A. S.; Phan, J.; Evdokimov, A. G.; Tropea, J. E.; Peters, H. K., III; Kapust, R. B.; Li, M.; Wlodawer, A.; Waugh, D. S.
- CS Center for Cancer Research, Macromolecular Crystallography Laboratory, National Cancer Institute at Frederick, Frederick, MD, 21702-1201, USA
- SO Russian Journal of Bioorganic Chemistry (Translation of Bioorganicheskaya

Khimiya) (2003), 29(5), 415-418 CODEN: RJBCET; ISSN: 1068-1620 PB MAIK Nauka/Interperiodica Publishing DT Journal English LA AB Tobacco etch virus cysteine protease (TEV protease) is widely used as a tool for the separation of recombinant target proteins from their fusion partners. Here, the crystal structures of 2 mutants of TEV protease, the active autolysis-resistant mutant TEV-S219D in complex with the proteolysis product, and the inactive mutant TEV-C151A in complex with a substrate, were determined at 1.8 and 2.2 Å resolution, resp. The active sites of both mutants, including their oxyanion holes, had identical structures. The C-terminal residues 217-221 of the enzyme were involved in formation of the binding pockets S3-S6. This indicated that the autolysis of the peptide bond Met-218-Ser-219 exerts a strong effect on the fine-tuning of the substrate in the enzyme active site, which results in a considerable decrease in the enzymic activity. RE.CNT 18 THERE ARE 18 CITED REFERENCES AVAILABLE FOR THIS RECORD ALL CITATIONS AVAILABLE IN THE RE FORMAT L7 ANSWER 8 OF 9 MEDLINE on STN DUPLICATE 6 2002733862 AN MEDITNE PubMed ID: 12377789 DM Structural basis for the substrate specificity of tobacco etch virus protease. Phan Jason; Zdanov Alexander; Evdokimov Artem G; Tropea Joseph E; Peters AII Howard K 3rd; Kapust Rachel B; Li Mi; Wlodawer Alexander; Waugh David S CS Macromolecular Crystallography Laboratory, Center for Cancer Research, NCI-Frederick, National Institutes of Health, Frederick, Maryland 21702-1201, USA. NC N01-CO-56000 (United States NCI) SO The Journal of biological chemistry, (2002 Dec 27) Vol. 277, No. 52, pp. 50564-72. Electronic Publication: 2002-10-10. Journal code: 2985121R. ISSN: 0021-9258. CY United States DТ (COMPARATIVE STUDY) Journal; Article; (JOURNAL ARTICLE) (RESEARCH SUPPORT, NON-U.S. GOV'T) (RESEARCH SUPPORT, U.S. GOV'T, P.H.S.) English LA FS Priority Journals OS PDB-1LVB; PDB-1LVM EM 200302 ED Entered STN: 27 Dec 2002 Last Updated on STN: 28 Feb 2003 Entered Medline: 27 Feb 2003 AB Because of its stringent sequence specificity, the 3C-type protease from tobacco etch virus (TEV) is frequently used to remove affinity tags from recombinant proteins. It is unclear, however, exactly how TEV protease recognizes its substrates with such high selectivity. The crystal structures of two TEV protease mutants, inactive C151A and autolysis-resistant \$219D, have now been solved at 2.2- and 1.8-A resolution as complexes with a substrate and product peptide, respectively. The enzyme does not appear to have been perturbed by the mutations in either structure, and the modes of binding of the product and substrate are virtually identical. Analysis of the protein-ligand interactions helps to delineate the structural determinants of substrate specificity and provides guidance for reengineering the enzyme to further

improve its utility for biotechnological applications.

.7 ANSWER 9 OF 9 MEDLINE on STN DUPLICATE 7

AN 2002329980 MEDLINE

DN PubMed ID: 12071693

TI A new vector for high-throughput, ligation-independent cloning encoding a tobacco etch virus protease cleavage site.

AU Stols Lucy; Gu Minyi; Dieckman Lynda; Raffen Rosemarie; Collart Frank R; Donnelly Mark I

CS Environmental Research Division, Argonne National Laboratory, Argonne, IL 60439, USA.

NC GM62414-01 (United States NIGMS)

SO Protein expression and purification, (2002 Jun) Vol. 25, No. 1, pp. 8-15. Journal code: 9101496. ISSN: 1046-5928.

Journal code: 9101496. ISSN: 1046 CY United States

DT Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, U.S. GOV'T, NON-P.H.S.)
(RESEARCH SUPPORT, U.S. GOV'T, P.H.S.)

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LA English

FS Priority Journals

EM 200212

ED Entered STN: 20 Jun 2002 Last Updated on STN: 28 Dec 2002 Entered Medline: 27 Dec 2002

To establish high-throughout methods for protein crystallography, all aspects of the production and analysis of protein crystals must be accelerated. Automated, plate-based methods for cloning, expression, and evaluation of target proteins will help researchers investigate the vast numbers of proteins now available from sequenced genomes. Ligation-independent cloning (LIC) is well suited to robotic cloning and expression, but few LIC vectors are available commercially. We have developed a new LIC vector, pMCSG7, that incorporates the tobacco etch virus (TEV) protease cleavage site into the leader sequence. This protease is highly specific and functions under a wide range of conditions. The new vector incorporates an N-terminal his-tag followed by the TEV protease recognition site and a SspI restriction site used for LIC. The vector functioned as expected, giving high cloning efficiencies and strong expression of proteins. Purification and cleavage of a target protein showed that the his-tag and the TEV cleavage site function properly. The protein was purified and cleaved under different conditions to simulate both plate-based screening methods and large-scale purifications for crystal production. The vector also includes a pair of adjacent, unique restriction sites that will allow insertion of additional modules between the his-tag and the cleavage site of the leader sequence to generate a family of vectors suitable for high-throughput production of proteins.

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